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CONFERENCE ON PROBLEMS OF GENERAL AND CELLULAR PHYSIOLOGY RELATING TO FERTILIZATION. I[1]

ACTIVATION OF EGGS, FERTILIZATION AND EARLY DEVELOPMENT AS AFFECTED BY ULTRAVIOLET RAYS

DR. ARTHUR C. GIESE

DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD UNIVERSITY

ULTRAVIOLET radiations, like many other agents, initiate artificial parthenogenesis in eggs. They also have other profound effects on eggs even when they do not initiate development. While sunlight may occasionally produce such effects in nature, it is unlikely, since the actinic radiations of sunlight are not very intense and penetrate only if the water is clear. Neither do such radiations enter more than a few millimeters into tissues, therefore gonads are safe from them. This is in striking contrast to the ionizing radiations such as X-rays which may penetrate tissues deeply and selectively destroy germinative or "embryonic" tissues such as the germinative epithelium, the blood-forming tissues and the stratum germinativum of the skin (see Giese, 1947, for references).

The studies which have been made with ultraviolet rays

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are therefore comparable to studies made with the effects of poisons, narcotics and anesthetics which do not occur naturally but which interfere with the normal course of development. The aim of such research is to gain an insight into the nature of fertilization and early development by interfering with the various processes concerned. If the data from a number of lines of such investigation are brought together, some features of fertilization and development are clarified.

I shall first discuss the initiation of development of eggs by ultraviolet light, then the effect of these rays on gametes and early development following fertilization.

Jacques Loeb (1914) was the first to initiate development in eggs with ultraviolet light. Using *Arbacia* and *Chaetopterus* and exposing them to the entire radiations of a mercury lamp in quartz, he observed that membranes were formed and that some of the eggs so activated went on to develop to the two- and the four-celled stage. Even if oxygen were eliminated by bubbling hydrogen through the suspension of eggs, the latter were activated. He found that a high temperature was deleterious, room temperature leading to disintegration, but that a low temperature such as 12° C. was satisfactory. When such activated eggs were put into hypotonic sea water they developed into larvae. Chaetopterus gave rise to larvae without cell division.

Lillie and Baskerville (1922*a* and *b*) studied the activating effect of these rays on starfish eggs. They were particularly interested in what they called partial activation, that is, the exposure to light decreased the time necessary for other agents such as heat or butyric acid to act. Heat of 33° C. was especially effective after ultraviolet, but was ineffective if applied before the irradiation.

Tschakhotine (1935*a* and *b*) was able to activate *Pholas* and sea urchin eggs by what he called ultraviolet puncture since he irradiated with a spot only 5 μ in diameter. The local spot irradiated shows either a bleb, or a depression, or one followed by the other. What interests us here is that the membrane first formed at the point of irradiation ex-

tends itself over the entire egg. He did not study the speed of formation, but this would be interesting since the action is so localized and in this respect more or less comparable to sperm puncture. Some eggs are activated by ultraviolet

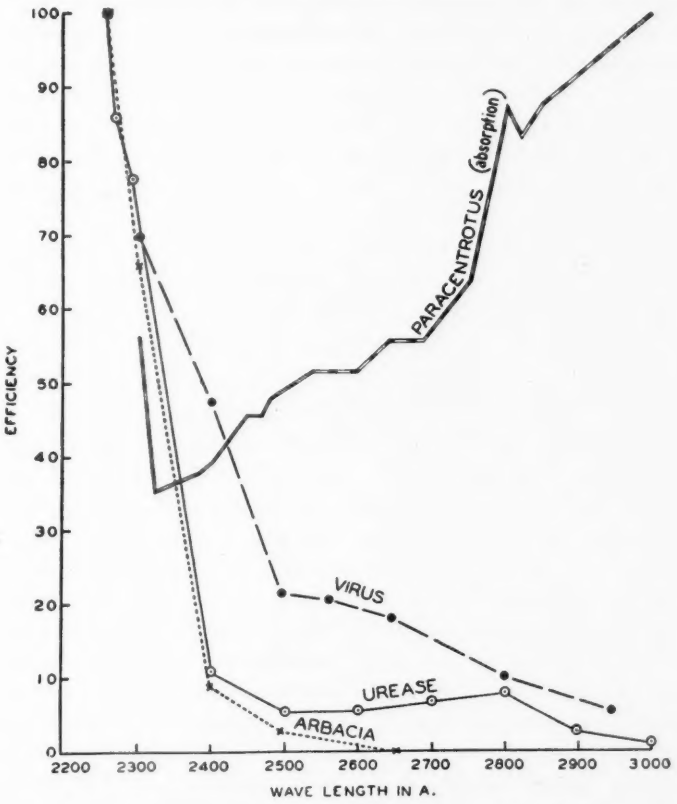

FIG. 1. Action spectrum for 50 per cent. activation of Arbacia eggs compared to destruction of urease and of virus and absorption by Paracentrotus eggs (from Hollaender, 1938).

light of λ 2537 Å (Giese, 1939) others not (Giese, 1938*a*).

Loeb (1914) surmised that only the short wavelengths of ultraviolet light produced activation, since insertion of a thin glass coverslip which cuts off wavelengths shorter than

3000 Å between the eggs and the source of light prevented activation of sea urchin eggs. Hollaender (1938) made a study on *Arbacia punctulata* at Woods Hole, using known dosages of monochromatic light. He found that large dosages of light at 2804 Å gave very little activation, and even at 2654 Å the effect was still only slight, but at shorter wavelengths the effects increased with decrease in wavelength. Activation is greater the shorter the wavelength over the span used. It is probable that even eggs reported unresponsive to ultraviolet rays would be susceptible if exposed to shorter wavelengths, but no one has yet made such a comparative study.

In Fig. 1 is shown what is known as an action spectrum, since it compares the action of different wavelengths of the spectrum on a particular biological reaction, in this case, activation. The action spectrum is thought to correspond fairly closely to the absorption spectrum of the substance being affected by the light. Such a deduction, it will be remembered, enabled Warburg to identify the nature of indophenol oxidase. However, such a deduction can be made only if the action spectrum has a characteristic structure by which it can be identified with the absorption spectrum of some material. As seen from Fig. 2 this activation action spectrum does not correspond to the absorption spectra of the nucleoproteins and the non-conjugated proteins which are the two commonest constituents of the cell. It is therefore necessary to attempt to match the action spectrum with the absorption spectrum of some of the other constituents of the cell: proteins lacking aromatic amino acids, lipids, carbohydrates, salts, and water. The latter three lack specific absorption (Caspersson, 1936). Both the lipids and the proteins lacking aromatic amino acids (such as the histones and protamines) have only a sort of general absorption in the short ultraviolet, increasing with decrease in wavelength (Caspersson, 1936). Both lipids and proteins occur in the membrane and Parpart and Dziemian have shown lipids and proteins in the ratio of 1:1.7. The protein in the stroma of the erythrocyte has been analyzed

(Beach, Erikson, Bernstein, Williams and Macy, 1939) and shown to contain both tyrosine and tryptophane. Such a protein would therefore show specific absorption. By the process of elimination it would appear that the action spectrum of activation corresponds most closely to the absorption by membrane lipids, but the evidence is not conclusive. The action spectrum of another effect of ultraviolet light,

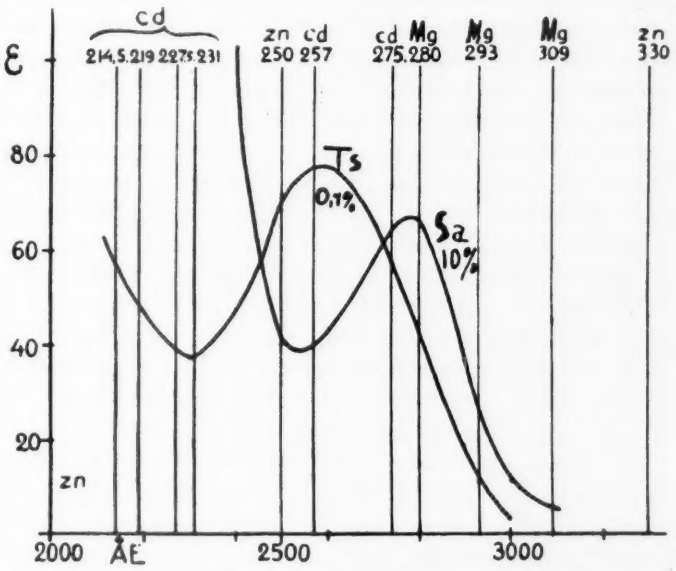

FIG. 2. Comparison of absorption by thymusnucleic acid and serum albumin. Note difference in absorption maxima as well as differences in relative absorption (from Caspersson, 1936).

hemolysis, which is thought to be an effect on the cell surface, resembles that of activation in that action is very slight even at λ 2804 Å, rising rapidly below 2530 Å (Sonne, 1929). This similarity makes more probable the supposition that both hemolysis and activation result from action on constituents of the membrane. One other surface effect suggests itself for study in comparison to activation and hemolysis—permeability. However, studies made by Reed

(1948) indicate no change in permeability of sea urchin eggs unless the cell is damaged, therefore no action spectrum could be determined.

Harvey and Hollaender (1937; 1938) have studied the activating effects of various monochromatic wavelengths of ultraviolet light on eggs stratified by centrifugation (10,000 × gravity) and on the fragments of such eggs centrifuged to the breaking point. They showed that the white halves are more sensitive to the rays than whole eggs. The red halves containing pigment are sensitive to all wavelengths from 2650–3050 Å. This indicates that not only the surface but also the pigment present deeper in the cell is active in initiating development. This series of experiments also confirms the already generally accepted view that activation is rather non-specific, and that the agents which bring it about set off some sort of trigger mechanism. However, the extent to which development follows such activation is dependent on the stimulus. Another conclusion which may be drawn from these experiments is that since the white halves only have the female pronucleus and since both halves may be activated by radiations, the nucleus is not necessary for activation.

That more than just the surface of the cells is affected by these radiations is shown by many cortical changes and by the alterations in the production of jelly and in the formation of the fertilization membrane after raying. Just (1933) believes that injury resulting from ultraviolet raying is largely cortical as judged by the changes in the jelly secretion in *Nereis* following raying. Spikes (1944) working with *Lytechinus* and Reed (1944) with *Strongylocentrotus* have described unilateral effects of ultraviolet in suppressing membrane formation on the irradiated side. Spikes reports that the cleavage plane passes through the axis of irradiation. Reed and Whitaker (1941) showed that polarized or unilateral plasmolysis occurs in *Fucus* treated unilaterally with ultraviolet light. Their interpretation is that the cytoplasm below the membrane is involved, being somewhat gelated by action of the rays. Their results suggest

that parts of the cell other than the membrane are affected by the radiations.

Much more clear-cut evidence for a general effect of ultraviolet light on eggs is indicated in the work in which the eggs are irradiated and then fertilized with unirradiated sperm, or *vice versa*. An example of the immediate effects of radiation on the early cleavages of eggs is shown in Fig. 3. The retardation increases with increase in dosage, but the increase is not linear, successive increments causing pro-

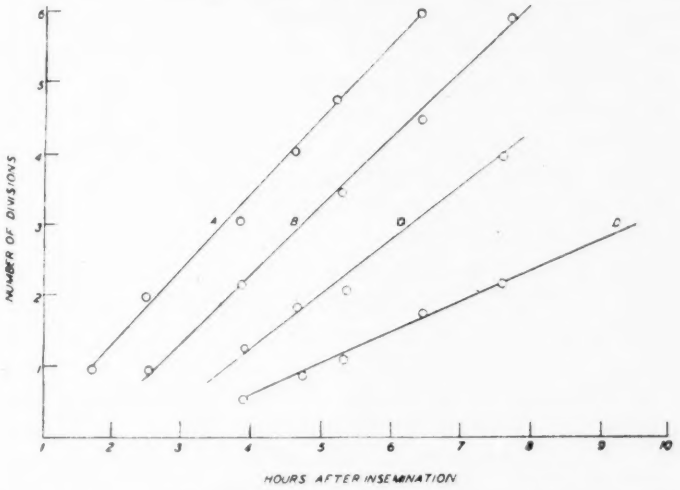

FIG. 3. Effect of λ 2804 Å on rate of division of eggs of the sea urchin, *Strongylocentrotus purpuratus* (from Giese, 1938*b*).

gressively lesser effects. Two questions arise: (1) Is the division of the egg merely retarded or (2) is development qualitatively altered? Data are available which show that if the embryos are examined at the blastula, gastrula or pluteus stages one finds that there is a progressive retardation but that the embryos so formed are to all appearances entirely normal, unless the dosage is excessive. In the latter case the blastulae are filled with cells of irregular size and may never differentiate (Giese, 1938*a* and *b*). One may say that small amounts of ultraviolet radiations merely

retard the divisions but excessive amounts interfere with normal development. Perhaps it is necessary to emphasize a point here which does not come out in the figures, namely, that the threshold for retardation is very high. Thus, from absorption data it is possible to show that the egg may absorb as much as 10^9 or 10^{10} quanta without showing any retardation in division even for short wavelengths. Only when ten times this amount has been absorbed are definite signs of retardation observed. If we assume for purposes of calculation that the egg contains only protein molecules of the size of albumin then the egg would have something of the order of 10^{12} molecules. Even if we are wrong by a factor of 10 or more in this calculation the amount of radiations which an egg can absorb without being affected is impressive. The fact that the larvae developing from retarded eggs not treated with excessive dosages still develop to all appearances normally indicates the remarkable recovery of irradiated eggs (Giese, 1938*a* and *b*).

Not all eggs respond in the same manner. Thus the eggs of Echinoderms show only retardation following smaller dosages and irregularities only after larger dosages. In the eggs of the mosaic type tested, for example, *Nereis, Urechis,* and *Chaetopterus* (Giese, 1946) there is a tendency for abnormalities to appear even after relatively small dosages. Another effect observed with the latter type of egg is that in some cases, *e.g., Nereis,* one may observe a localized effect on one side of the unilaterally irradiated egg and later on the embryo.

The action spectrum for retardation of division by irradiation with ultraviolet light has been worked out in the case of *Strongylocentrotus purpuratus* (Giese, 1946). As seen in Fig. 4 the action spectrum for retardation of the irradiated egg is a fair match for the absorption spectrum of albumin or globulin or similar proteins (Fig. 2). The maximum effect appears where such proteins have a maximum, namely, 2800 Å. This suggests that these proteins are affected and their transformation results in the retardation of division. The action spectrum demonstrates clearly that

the substance affected which leads to the retardation of division is different from that which is affected in activation and artificial parthenogenesis. The egg is therefore affected by ultraviolet light in more than one locus in the cell and in more than one way. Although this action spectrum does not implicate the nucleus or the nucleoproteins, there is evidence that the nucleus is affected. Studies by Stevens

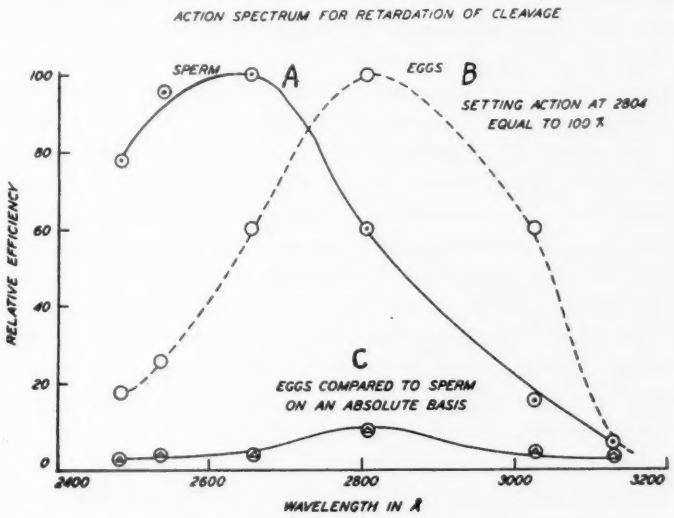

FIG. 4. Action spectra for sperm and eggs of *Strongylocentrotus purpuratus* (from Giese, 1946).

(1909) and later by Schleip (1923) on *Ascaris* eggs show that when whole eggs are irradiated a wide range of chromosomal abnormalities follow: thus clumping, excessive chromatin diminution, lagging of chromosomes and thickening of the membranes were observed among other things. Even if the nucleus was screened and only the cytoplasm was irradiated, various abnormalities appeared similar to those observed when the entire egg was irradiated. However, larger dosages were required. This suggests that cytoplasmic injury may lead to formation of toxic materials that affect the chromosomes which have not been rayed.

Sperm directly rayed are much more susceptible than eggs. This is easily demonstrated by inseminating untreated eggs with irradiated sperm and studying the delay in cell division. A graded series is obtained with dosage. If the effects on eggs and sperm are compared on the basis of the light actually absorbed by each, then only one millionth the energy at λ 2654 Å is required to produce effects on the sperm as on the egg. This indicates a sensitivity of an entirely different order of magnitude. If the effectiveness on sperm of different wavelengths is compared, an action spectrum is obtained which corresponds more closely to the absorption spectrum of nucleoproteins (Giese, 1946) than to any other known cellular constituent. This is not surprising in view of the fact that between 60 to 80 per cent. of the contents of the sperm are nucleoproteins. The sperm nucleus is quickly protected once it penetrates the egg and the susceptibility of fertilized eggs is similar to the unfertilized.

Perhaps of all the experiments on retardation of division of eggs the most striking are those on suppression of division of eggs by irradiation. Suppression of cleavage is possible within the first thirty minutes after insemination. After this, larger and larger dosages are necessary. Just before division even very large dosages are ineffective; however, cleavage is followed by cytolysis. The cell is set to divide and even excessive damage no longer prevents it.

Just what synthetic reactions in the cell are affected by ultraviolet radiations is not clear, but an inkling has been gained from studies involving ultraviolet photography and Feulgen staining. Caspersson and his coworkers have shown that desoxyribose nucleic acid is synthesized during its mitotic cycle and the desoxyribose nucleic acid rises during the prophases and reaches its highest concentration during the metaphase. It falls during the anaphase and reaches its minimum concentration during the interphase. The nucleolus contains ribosenucleic acid and perhaps is the center of formation of this compound. It disappears in many cases during the formation of the chromosomes and

reappears before interphase. Parallel with the nucleic acid cycle is the cycle of complex and simple proteins. When the nucleoproteins of the desoxyribose type are at a maximum, the complex proteins containing the aromatic groups (which can be studied spectrophotometrically) are at a minimum and the histones (without aromatic nuclei) are at a maximum.

This cyclic change, the exact significance of which is not at present clear, is interfered with by radiations. At least it has been noted in a number of instances that injured or dying cells absorb ultraviolet light more strongly (Luyet and Gehenio, 1936), and Brumberg and Larionow (1946) claim that one can not see the nucleus as distinct from the cytoplasm in photographs taken at all wavelengths between 2540–2750 except after prolonged irradiation. They claim that the radiations have injured the cells so that the photographs show dying or dead cell constituents. Irradiation of cells may lead to an accumulation of ultraviolet absorbing materials, as shown by Loofbourow (1948). Loofbourow presents evidence which indicates that cells deeply injured by ultraviolet radiations lose, through their membranes, not only nucleotides but also vitamin B factors and amino acids, which filtered free of cells stimulate growth and respiration in other unirradiated cells. Spectrophotometric analyses show that the nucleotides which appear are present in proportions other than would result from a mere breakdown of the nucleoproteins of the cells. This suggests synthesis of certain of these nucleotides by the injured cells. It is possible that the darkening of the injured cells is due to an accumulation of nucleotides since ultraviolet light does not discriminate between the nucleotides and nucleic acids, the absorption being largely dependent on the quantity of purine or pyrimidine bases present in the compounds.

It should be possible to study some of the chemical changes which occur in unfertilized eggs activated by ultraviolet by the photographic and spectrophotometric method. Harvey and Lavin (1944) have made a study of the unfertilized and the fertilized Arbacia egg up to just before cleav-

age. They show that nucleoproteins are present throughout the cytoplasm and that they are concentrated in the nucleolus and the chromatin at the germinal vesicle stage. As the germinal vesicle breaks down the nucleoprotein increases in the nucleus, but the amount in the cytoplasm does not change much. Perhaps a slightly greater concentration appears about the nuclear membrane which is thought by some to be the seat of formation of these materials. The cyclic change in constituents after irradiation has not been studied in any case.

The following general conclusions may be drawn from the account presented:

(1) The mature egg ready for fertilization seems to need only a trigger action of some change to set off activation. Ultraviolet light may act as the trigger but more nearly normal development is obtained if the radiation is followed by a secondary treatment such as heat or hypertonic sea water.

(2) The ultraviolet action spectrum suggests that the material affected by these rays is probably in the membrane and is either lipid or protein devoid of aromatic amino acids, or both. It is probably not an effect on nucleoproteins or on the common unconjugated proteins.

(3) The effect of ultraviolet radiations is deleterious and never beneficial to both sperm and eggs, sperm being more sensitive than eggs.

(4) So far as suppression of cleavage is concerned the egg is more sensitive in the early stages than later. It appears that once certain processes have been set in motion no amount of radiation short of complete cytolysis will stop them.

(5) The threshold dosage which an egg can tolerate before it is injured is very high. A fair proportion of the protein molecules will have absorbed some energy before any signs of retardation show up.

(6) Some evidence indicates that ultraviolet light interferes with the manufacture or conversion of nucleoprotein. Absorption studies suggest that nucleoproteins or nucleo-

tides accumulate in the irradiated cell which becomes more opaque to these radiations.

(7) There is as yet no evidence for parthenogenesis produced by use of ultraviolet treated sperm; if sperm are introduced, no matter how battered they are, they always seem to combine with the egg nuclei and at least influence the division (Giese, 1946).

(8) Ultraviolet light, like so many other interfering agents, is not a specific tool for stopping a particular process in the cell. General injury to the cells is indicated by the fact that different action spectra are obtained for activation and retardation of division.

(9) While ultraviolet light used as a single technique may not be so promising for further studies on fertilization and development, it may be that small dosages followed by other agencies such as heat (Lillie and Baskerville, 1922*a, b*) might be a fruitful field of study. The reversal of ultraviolet effects by visible radiation concurrently applied or subsequently applied may also be interesting (Whitaker, 1942) and has recently been studied on viruses and bacteria (Kelner, 1949).

LITERATURE CITED

Beach, E. R., Bn. N. Erikson, S. S. Bernstein, H. H. Williams and I. G. Macy
1939. *Jour. Biol. Chem.*, 128: 339.

Brumberg, E. M., and L. T. Larionow
1946. *Nature*, 158: 663–664.

Caspersson, T.
1936. *Skand. Archiv. f. Physiol.*, Suppl. No. 8 to Vol. 73: 1–151.

De Robertis, E. D. P., W. W. Nowinski and F. A. Saez
1948. "General Cytology." Ch. 6, 119–133.

Giese, A. C.
1938*a*. *Biol. Bull.*, 74: 330–341.
1938*b*. *Biol. Bull.*, 75: 258–265.
1939. *Physiol. Zool.*, 12: 319–328.
1945. *Physiol. Zool.*, 18: 223–250.
1946. *Biol. Bull.*, 91: 81–87.
1947. *Quart. Rev. Biol.*, 22: 253–282.

Harvey, E. B., and A. Hollaender
1937. *Biol. Bull.*, 73: 365.
1938. *Biol. Bull.*, 75: 258–265.

Harvey, E. B., and G. Lavin
1944. *Biol. Bull.*, 86: 163–168.
Hollaender, A.
1938. *Biol. Bull.*, 75: 248–257.
Just, E. E.
1933. *Archiv. Entwicklungs.*, 130: 495–516.
Kelner, A.
1949. Proc. Natl. Acad. Sci., 35: 73–79.
Lillie, R. S., and M. L. Baskerville
1922*a*. *Am. J. Physiol.*, 61: 57–71.
1922*b*. *Am. J. Physiol.*, 61: 272–288.
Loeb, J.
1914. *Science*, 40: 680–681.
Loofbourow, J. R.
1948. *Growth Symposium*, 12: 75–149.
Luyet, B., and P. M. Gehenio.
1936. *Biodynamica*, No. 11: 1–8.
Parpart, A. K., and A. Dziemian
1940. Cold Spring Harbor Symp. Quant. Biol., 8: 17– .
Reed, E. A.
1944. *Anat. Rec.*, 87: 39.
1948. *Jour. Cell. Comp. Physiol.*, 31: 261–280.
Reed, E. A., and D. M. Whitaker
1941. *Jour. Cell. Comp. Physiol.*, 18: 329–338.
Schliep, W.
1923. *Arch. Zellforschung*, 17: 289–367.
Sonne, C.
1929. *Arch. Physical Therapy, X-rays, Radium*, 10: 239–252.
Spikes, J.
1944. *Jour. Exp. Zool.*, 95: 89–102.
Stevens, N. M.
1909. *Arch. Entwicklungsmech.*, 27: 622–639.
Tschakhotine, S.
1935*a*. *Compt. Rend. Soc. Biol.*, 119: 1394.
1935*b*. *Compt. Rend. Soc. Biol.*, 120: 714.

Discussion

Chairman Engle: Thank you, Dr. Giese. The work was not only carefully done and presented, but you did it in exactly thirty minutes. It is now open for discussion.

Dr. William E. Berg: Is it possible to determine the degree of absorption by the cytoplasm at a particular wavelength? What would be the absorption by the first millimeter of cytoplasm of the egg?

Dr. Giese: Such measurements have been tried on sea urchin eggs by Vles. The egg being a lens makes a difficult

system to study. However, approximations may be obtained in the following way: We take a suspension of eggs and determine the amount of light of a given wavelength passing through, but there are considerable scattering errors. For this we can correct by measuring visible "absorption," which is scattering since the egg is colorless, and extrapolating into the ultraviolet. At wavelength 2537 Å practically all the light is taken up by an egg so that one egg thickness is sufficient to absorb all incident light. Longer wavelengths are absorbed less completely—the absorption being about 76 per cent. at 2804 Å. Absorption falls off rapidly to about 25 per cent. in the visible. This correction might be applied to the ultraviolet, multiplying by a factor obtained from the equation for scattering of light by particles. Approximately half the ultraviolet light wave would appear to be scattered.

DR. A. M. SCHECHTMAN: I would like to ask whether you have tested the effect of various dosages on eggs after cleavage has started. In the literature in embryology one finds quite a number of references to stimulation by low doses of ultraviolet rays. I wonder whether after cleavage had begun, one might notice a stimulatory effect rather than only an inhibitory effect.

DR. GIESE: The only clear-cut case of stimulation I can remember of eggs is that described by Chase on several species of eggs studied at the Hopkins Marine Station. Irradiation was performed before fertilization, the polar bodies appeared a little early, and the first cleavage was slightly stimulated, and I don't really know whether it is truly significant. I don't know of any other careful studies in which any significant stimulation was observed.

DR. SCHECHTMAN: Would you consider it a safe statement to say that in normally activated eggs or eggs activated by artificial means, on the whole, ultraviolet treatment, in any dosage, would have a retarding or an injurious effect?

DR. GIESE: In general I think you can make that statement. I have tabulated all cases in the literature where

stimulation was reported. The best analyzed is that of Loofbourow (see *Growth Symposium*, 12: 75–149, 1948). In that case the stimulation results from growth factors, members of the Vitamin B series, amino acids and nucleotides which leak out of the ultraviolet injured cells. The filtrates have been analyzed chemically and duplicated with artificial sources containing the same concentrations with the same stimulating effect.

DR. SCHECHTMAN: In the forms with which you have worked there is relatively no growth in the stages that are involved whereas working with the chick or tissue cultures one has both a growth process and differentiation which might give us two types of effects with which to deal.

DR. GIESE: In Loofbourow's laboratory a study was made with tissue culture, but it proved too complicated to work with and most of the work has been done with yeast cells. However, Loofbourow's associates did get the same sort of stimulatory effects with chick tissue culture. There is also one other good case of great stimulation reported for Stichicoccus but it is a genetic effect. In all other cases reported for the protozoa, inadequately controlled cultures were used so that I don't think any conclusion is justified.

DR. TYLER: Following the report of the reversal of the lethal effect of ultraviolet radiation by visible light in bacteria we have performed similar experiments with sea urchin sperm. We have run five sets of experiments. The first four were increasingly positive. The fifth one was quite negative. At present we are quite undecided about the ability of visible light to reverse the effect of ultraviolet on the sea urchin sperm.

DR. LINUS PAULING (California Institute of Technology): I think it would be interesting if you were getting effects that depended on only a few quanta. Since the sperm is so much smaller than the egg, and the efficiency of irradiating sperm is ten times as great, it would appear that, on the basis of energy per unit area, your figure of 10^9 for an egg becomes 10^2 or 10^3 for a sperm.

DR. GIESE: If one determines the energy absorbed by the

sperm, again with these rough corrections I mentioned, it turns out that the sperm is a million times more sensitive than the egg.

DR. PAULING: Aren't the observations good enough so one can determine the number of quanta involved from the fluctuation theory?

DR. GIESE: How accurate would they have to be? These scattering correction factors introduce a good deal of uncertainty.

DR. PAULING: You would observe the effect for an individual fertilized egg once in a while after very small amounts of irradiation, and then with increasing frequency with larger amounts. In these fluctuations, if the effect is clean-cut, you don't need to average data. You look at each egg and see what has happened.

DR. GIESE: We are always working with populations of eggs of 200 or so. Since they divide synchronously, and since the threshold is so high, I rather doubt that you can find any effects after a small dosage. If the sperms are irradiated with a small dosage, no effect on eggs fertilized with such sperm can be seen.

DR. PAULING: I think there is still a possibility of observing the effect of a small number of quanta if the normal behavior is uniform enough to permit you to recognize the abnormalities individually.

DR. DELBRUCK: The crucial point is, can you determine the fraction of effect or non-effect on sperm to the following categories: less than one per cent., between ten and ninety per cent., to within one-tenth of one hundred per cent.?

DR. GIESE: I haven't made any attempt of that sort. I think it could be done.

CHAIRMAN ENGLE: Those are very important points raised, particularly this whole business of relative absorption of the sperm and egg, one having a high percentage of nucleoprotein and the other being high in ordinary proteins.

DR. DANIEL PEASE: A problem which has interested me considerably is the mechanism of sperm penetration through the egg membrane. There is plenty of reason, of course, to

believe that the gel of the egg cortex has a good deal to do with the penetration. In view of the radiation effects upon cytoplasm it would seem to me pertinent to study the effect of radiation on the actual formation and development of the fertilization cone. It may be particularly sensitive to radiation effects. I suppose it could be done in an egg which has a cone which persists for maybe fifteen minutes or so. I don't know of any such experiments in the literature, though, do you?

DR. GIESE: I don't know of any study of that sort. Usually the eggs fertilized perfectly well, though the sperm or the egg had been irradiated.

DR. PEASE: I suppose it is quite possible that you would need massive doses to produce a block.

DR. GIESE: The ultraviolet treated sperm always seems to participate in the formation of a zygotic nucleus as far as we know. This is in contrast to the sharp effect Packard got after ionizing radiations. In that case a sperm given a large enough dose would still enter and activate the egg, but it would then become inactive.

DR. EDWARD L. CHAMBERS: I am interested to know if radiation produces a reversible gelation of the surface of the egg. This might be studied with unilateral radiation.

DR. GIESE: Dr Spikes has done such experiments and I shall refer your question to him.

DR. JOHN D. SPIKES: I don't know whether there is much evidence for reversal. Apparently it only takes small amounts of radiation to have some sort of gelling effect.

DR. SPIKES: Is there a difference in the effects of heat and visible light following ultraviolet exposure of eggs or sperm?

DR. GIESE: I think heat and visible light would have different effects. Experiments with heat indicate that even extremely small amounts of radiation sensitize the protoplasm. For example, Paramecium irradiated with a small dosage at 2480 Å is unaffected in any detectable way. The animals go on dividing as if nothing had happened. Yet with a dosage of heat which does not injure controls the animals are killed. Comparing different wavelengths, one

obtains an action spectrum which resembles absorption by serum albumin. Dr. Pauling has suggested that perhaps the molecules of enzymes affected by ultraviolet light which would remain oriented and go on functioning even after they absorb the light, on application of higher temperatures, become disoriented with respect to each other and death results. Visible light, on the other hand, is thought to reverse ultraviolet damage, as Dr. Tyler mentioned.

DR. ERWIN HAAS: I also believe that light and heat would have quite different effects, especially since it is the visible light which seems to be active in reversing the destructive action of ultraviolet irradiation. If it could be shown that blue light was most effective one might suspect that growth factors are produced here by photochemical decomposition of yellow enzymes which exhibit their maximum light absorption in the blue region of the spectrum. The formation of a photo derivative of the prosthetic group of yellow enzymes by irradiation with visible light of low intensity has been discovered by Warburg and Christian and has been determined more quantitatively in 1935 by Theorell.

CHAIRMAN ENGLE: One of the advantages of this kind of a meeting is that we can have all day to discuss these things, and many of the discussions are most pertinent when you button-hole the speaker in the corridor and get him to tell what he thinks about it. I think we should keep reasonably to the schedule and proceed to the next presentation by Edward Novitski, California Institute of Technology, on "Action of X-rays at Low Temperatures on the Gametes of Drosophila."

ACTION OF X-RAYS AT LOW TEMPERATURES ON THE GAMETES OF DROSOPHILA[1]

DR. EDWARD NOVITSKI

California Institute of Technology

Probably more is known about the effects of irradiation on Drosophila than in any other organism. Here the effects usually studied are not those which come under the general heading of physiological effects but rather those of gene and chromosomal mutation. Undoubtedly there are some basic similarities of these two superficially different kinds of effects; the subject of this presentation is one such similarity, the relation of the X-ray induced mutation rate to one such physiological state, temperature.

The work with Drosophila has yielded a reasonable thermodynamic concept of the process of mutation. According to this theory, the energy of the secondary electrons produced by the X-rays, which is far in excess of the energy required to break the most stable chemical bond, may dissipate itself eventually as a chemical change in the gene itself, and that change may be detectable as a gene mutation. As a corollary to this, it is obvious that the temperature of the medium surrounding the gene and the chromosomes at the time of irradiation should have little if any effect because the increment of thermal energy in those limits where work with living material is feasible is negligible in comparison with the energy released by absorption of the radiation. However, if a temperature effect were to exist one might suppose for simple thermodynamic reasons that genes

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would mutate somewhat more readily at high than at low temperatures.

The existence of an effect of temperature during irradiation has been tested for by Drosophila workers several times, and the results have been conflicting. Oddly enough, the positive results which have been reported have been in just the opposite direction to that expected; that is, the effectiveness of the irradiation appeared to increase at the lower temperature. Since this conflicts with the above notion concerning the mutation process, additional data have been collected bearing on the point.

The class of mutation studied here is that of sex-linked lethals in Drosophila, which is susceptible to an objective quantitative analysis. Without going into description of the mechanical procedure of analyzing for this type of mutation, it may be mentioned briefly that these lethal mutations kill the animal carrying them if a normal compensating allele is not present.

The first phase of the work involved the study of the effects of very low temperatures applied during irradiation, compared with the effects of the same X-ray dose at higher temperatures. For this study, it was found possible to treat Drosophila at temperature levels as low as $-5°$ C. It was found that the mutation rate per roentgen unit did increase at the lower temperatures, the magnitude of the increase being about 75 per cent. Without presenting the raw data here, it may be stated simply that the differences between the two sets were such that they would have occurred as random variations in a single normally distributed population in only once out of five hundred trials. Similar highly significant differences apply to the results described below.

It was found that the mutation rate could be increased not only by decreasing the temperature during irradiation, but also by decreasing the temperature *after* the irradiation treatment. In fact, the one experiment which gave the greatest difference between the sets at high and low temperatures was one of this sort.

On the basis of this information, certain tentative con-

clusions can be drawn: (1) that class of induced changes in Drosophila known as induced lethal mutations, known to consist of a variety of different types of gene and chromosomal alterations, does not fit into the thermodynamic concept of the process of gene mutation. It may be questioned whether any of the so-called "gene mutations" in Drosophila are evoked by a chemical reaction that takes place at or near the gene immediately after the absorption of the individual quantum. This question is fortunately open to a direct attack. (2) Since the reactive agents produced by the absorption of an X-ray quantum are generally considered to be available for chemical transformations only a fraction of a minute after the impact of the quantum (although recent data from the study of radio-chemical reactions suggest that this may not be completely true) the effect of the temperature shock after irradiation in increasing the mutation rate may perhaps be referred back to the mutation process itself. Thus the lower temperatures might be inhibiting some sort of "back-reaction," which at higher temperatures returns a certain proportion of potential mutations to their original state or to one not detectable as a mutation. This phenomenon has been observed for other types of radiation changes, as chromosomal breaks.

Discussion

Mr. George E. MacGinitie: (California Institute of Technology): Were these temperatures lowered and raised at the same rates in all experiments?

Dr. Novitski: Yes. There are so many variables in the problem that that is one thing we tried to keep constant. The rate of lowering was brought about by exposure to a cold air blast and the temperature rise by ordinary exposure to room temperature so that the lowering was much faster than the rise.

Dr. Spikes: If I might be permitted to shift the emphasis from the genetic viewpoint for a moment, can you tell anything about the relative fertilizing power of the sperm after these three treatments? That is, where they received

the same quantity of radiation but are treated differently. Is there any way of telling if you have a difference in fertilizing power of the sperm?

DR. NOVITSKI: Ordinarily the supply of sperm is far in excess of the demand, and if any sperm are knocked out because of the lost fertilizing capacity, they are undoubtedly replaced by those which can fertilize the egg. There is an effect on the viability of the individuals which result from fertilization with sperm which have been treated in this way, but here the effect is a chromosomal one and not physiological. There are some rather interesting results which bear on the more general problem of fertility. When we first started, it was decided that a temperature as low as possible should be used provided it did not interfere with fertility. A fairly large series was made up with flies, both female and male, subjected to low temperatures for quite a long period of time. It was discovered as long as a male or female survived it could then be mated and would show no infertility. We then took fertilized females and subjected them to low temperature during the irradiation. Oddly enough the animals proved to be sterile, and a little work on the point showed that what was happening was that the sperm which were stored inside the females were very susceptible to the cold treatment, whereas the same treatment had no effect on the sperm in the males or on the ova of the females.

DR. NORMAN H. HOROWITZ (California Institute of Technology): Do you know anything about the cytological effects of these treatments?

DR. NOVITSKI: That involves the analysis of large numbers of different lethals to determine whether or not in the cold treatment some one type was favored over another. I think I was deliberately vague on the definition of a lethal mutation; probably a large number of them are associated with chromosomal aberrations. It is known that a low temperature treatment increases the frequency of X-ray induced chromosomal abnormalities, but the correlation with lethals remains to be done.

DR. RAY D. OWEN (California Institute of Technology): Do you have any X-ray dosage data connected with this temperature treatment? If the situation were different from that normally studied without temperature treatment, might one detect it in terms of dosage curves?

DR. NOVITSKI: The dosage data for the two doses used, 1800 and 3600 r-units, show that the increment of effect after treatment at low temperatures is approximately proportional to the effect without such treatment. This relation does not appear to give any specific information about the nature of the differences in the two cases.

CHAIRMAN ENGLE: I take it nobody has studied cytologically the effects of a given standard dosage plus low temperatures on these flies to see if, at a certain temperature, you get differential destruction or disturbance of stages of spermatogenesis.

DR. NOVITSKI: No, as far as I know that has not been done. Drosophila is probably not the best animal to do it on.

DR. PAULING: Perhaps I should draw something on the board. I have been trying to understand how a mutation occurs and trying to get evidence about it by the simple method of asking geneticists what was going on. The problem that bothers me in general about reproduction is that I would not expect the use of a template for the formation of a replica to be perfect, 100 per cent. perfect. In the formation of antibodies with an antigen as the template great fluctuations are observed in the nature of the product, so a gene too, I would think, would not produce an exact replica of itself but something in the neighborhood; then, in the course of a long period of time a gene would settle down to such a configuration as would be most effective in producing a replica of itself. I might represent that by a curve of energy, although the property is somewhat different from energy (Fig. 1). Nevertheless, energy or stability is related to this property of being able to produce a replica of itself. A gene in the wild type of an organism might be represented by a point, A, near the mini-

mum of this curve. Although there are fluctuations when such a gene produces a replica, A′ or A″, in the next mitosis, there is a good chance that a better replica of the original gene will be produced, nearer the bottom of the curve. The gene would thus tend to stay in the valley. If we continue drawing the curve, there may be another valley over here somewhere at B, and on irradiation perhaps you get not by cell division but by damage to the gene a changed gene represented by a point such as C. When the next cell division occurs in the process of reproduction you have perhaps

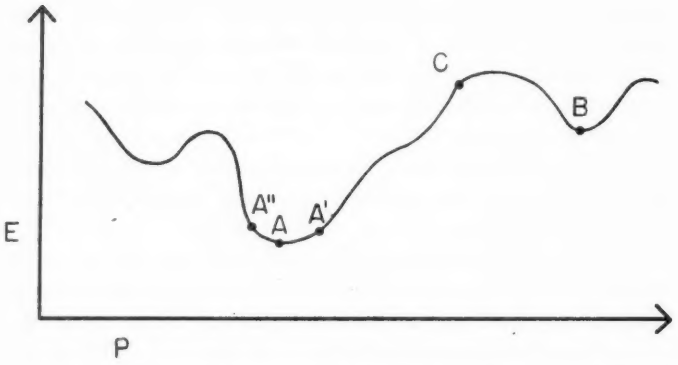

FIG. 1. Curve to illustrate manner of occurrence of a mutation. *E*, energy or other property related to self-duplicating power of a gene; *P*, parameter describing configuration of the gene. See discussion by Pauling in text.

nearly equal chance of this point moving over toward B or of moving back toward A. There is some chance of getting over the top of the hill and starting down to B, which would represent the lethal mutation, hence there would be, after the damage due to irradiation, a probability factor as to which direction the gene would go. A certain number of the new genes would be mutants, and a certain number would be the normal ones as a result of cell division.

Now what would be expected to happen without cell division? We have the damaged molecule, C, in a sort of unstable state where it has been left after the impact of the

radiation. Thermal agitation would have the effect on it of tending to cause it to " mosey " down the hill toward the normal type A. Obviously, the resultant effect of change of temperature would be determined by the relative rates of the reactions of repair of the damaged gene by thermal agitation and of cell division. It seems to me that this idea offers the possibility of a rational explanation of these experiments and perhaps of other radiation experiments.

DR. NOVITSKI: When you say that it " moseys " down the hill, you imply that there is a fairly long time period involved?

DR. PAULING: The time required to get down this hill would be of the order of magnitude of the time of molecular motion.

DR. NOVITSKI: I have experiments underway now which will give indications of the times involved at which this hypothetical " back reaction " can occur.

DR. ERIK ZEUTHEN: It is interesting that a sudden decrease in temperature just after the radiation is over may cause genetic damage, which might otherwise have been repaired, to show up. In the field of comparative physiology it has been shown that the immediate effect of a steep drop in body temperature is a " too low " energy metabolism. I say " too low " because during the following hours or days the energy metabolism again increases and becomes steady on a much higher level—at the same low temperature. Do you think this has any bearing on your problem? Metabolic energy might be involved in a repair process.

DR. NOVITSKI: I think that is a very good point, but our results indicate that if the period of time at the low temperature after the irradiation is of the order of half an hour, it produces much less effect than if the animals are kept down for fourteen hours.

DR. PEASE: I like your general notion, if I understand you correctly, that probably gross chromosome aberrations are most important in this effect. I wonder again if we can't interpret this in terms of gelation. I am thinking of a few data I never published which I collected years back

on the effects of temperature coupled with high hydrostatic pressures. The data were not very significant until you got down to zero degrees or very close to it, but then, all of a sudden, the plasmagels became unduly sensitive to pressure. Apparently there was some sort of a complex and subtle effect on, shall we say, the steric relations of the protein molecules. Conceivably this would make them particularly sensitive to irradiation.

DR. NOVITSKI: That could be one of the finer aspects of the problem which I broadly termed a "physiological effect." I would like to point out that as far as chromosomal breakage itself is concerned, there has been a good deal of argument about the relation of this sort of mutation to gross chromosomal changes, and Fano has provided a very convincing argument to show that these lethal mutations should not be interpreted as the results of chromosomal breakage, but must have another origin, in part, at least.

DR. GIESE: I thought it might be interesting to point out a contrast here between the effect of irradiation and temperature on mutation and on activation. If one keeps irradiated unfertilized eggs at room temperatures most of them die; only at low temperatures does one obtain activation. Destructive processes are accelerated at high temperatures, death being the result. With mutation, on the other hand, the high temperature results in repair. However, in sea-urchin eggs repair is a result of active metabolism. Thus irradiated sea-urchin eggs can be stored for long periods of time after radiation. If they are inseminated at different times, the retardation per given dose of radiation is not decreased and apparently the repair process, whatever it is, is extremely slow, not showing up even eight hours after irradiation. In other words, until fertilization is accomplished and the various reactions which occur in the developing egg are stated, there doesn't seem to be much repair. The same thing can be seen in experiments with protozoa. Recovery from the effects of radiation is gradual and occurs only if the animals are fed and carrying on active metabolism.

DR. SPIKES: In confirmation of what Dr. Giese said, we found that the effect of ultraviolet light in suppressing the fertilization membrane persists for a long time after ultraviolet radiation. We kept the unfertilized eggs sixty-five or seventy hours and apparently there was no recovery at all from ultraviolet light.

DR. SCHECHTMAN: We have quite a number of people interested in immunology here. I would like to ask whether, in the case of the rabbit or another animal if anybody present has heard of studies indicating that changes in the temperature of an animal result in qualitative differences in the antibodies, or are there only quantitative differences? I wonder if anyone knows of any studies which have a bearing on this idea.

DR. EMERSON: There was some work done by a student in this laboratory using fish which could be kept at low temperatures. More recent work has been done on fish and frogs. That has all been done on the quantities of antibodies and not characteristics. The most recent work showed if fish are kept at a low enough temperature they produce no antibodies. If they are kept a number of weeks and then brought back to higher temperatures, they produce antibodies as they would have to start with.

PROPERTIES OF FERTILIZIN AND RELATED SUBSTANCES OF EGGS AND SPERM OF MARINE ANIMALS[1]

DR. ALBERT TYLER

KERCKHOFF LABORATORIES OF BIOLOGY,
CALIFORNIA INSTITUTE OF TECHNOLOGY

INTRODUCTION

SINCE I have recently published a rather detailed review of the work on fertilizin and related substances (Tyler, 1948) I shall discuss here only a few points on which we have some additional information. Some of the present audience may not be acquainted with this work and others may not recall very well the general nature of the reactions with which we are concerned. So I shall first review briefly, with illustrations, the action of these substances and indicate what role they are considered to play in the process of fertilization. Following this, I shall discuss their relation to the tissue- and species-specificity of fertilization, their location in the gametes and finally I shall present some information concerning their chemical properties.

F. R. Lillie (1913) demonstrated that the egg-waters of the sea urchin Arbacia and of the polychaet annelid Nereis caused an agglutination of the homologous spermatozoa and used the term fertilizin to designate the active agent in the egg-water. Fertilizins have since been obtained from eggs of various species of invertebrates and have recently been reported to occur in eggs of vertebrates too (see review by Tyler, 1948). We have demonstrated some time ago that

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fertilizin is derived from the gelatinous coat of the egg of the sea urchin and of other animals. The gelatinous coat slowly goes into solution as the eggs stand in water and yields a solution of fertilizin. Various extraction procedures will dissolve the gelatinous coat rapidly and give concentrated solutions of fertilizin. Our tests have shown no other substance of large molecular size, apart from fertilizin, to be present in the gelatinous coat.

The substance on the sperm with which fertilizin reacts is termed antifertilizin. Some years ago Frank (1939) and I (1939) were able to extract this from sperm of the sea urchin and of other animals. There is also, within the egg, a substance that has properties similar to the antifertilizin which is obtained from sperm. The finding (Tyler, 1940) of such a substance within the egg that can react with a substance on the surface of the egg, and similar findings with other types of cells, have given rise to a so-called auto-antibody theory of cell structure, growth, and differentiation. This concept has been previously presented in some detail (Tyler, 1947) and will not be reviewed here. Another substance which is obtained from the sperm is a lytic agent. We have extracted this from sperm of various mollusks. Similar lytic agents have been obtained from sperm of fish, frogs, and mammals. The substance called hyaluronidase, which is derived from sperm of mammals, belongs to this general group.

Action of Fertilizin, Antifertilizin, and Lysin

Fig. 1 illustrates the agglutination reaction with sperm of the keyhole limpet. Fig. 1*a* shows a Syracuse dish with a suspension of untreated sperm. Fig. 1*b*, 1*c* and 1*d* show the reaction at 15 seconds, 30 seconds and ten minutes after addition of a solution of the fertilizin obtained from the eggs of this species. The sperm agglutinate in quite compact masses.

Fig. 2 shows the microscopic appearance of some of the agglutinates. In dense suspensions spherical masses of sperm are usually formed. Very often each sphere is sur-

rounded by a complete or incomplete shell of sperm heads attached to the main mass by their tails.

Fig. 3 shows higher power photographs of small agglutinates. I am always intrigued by Fig. 3*b* which shows a

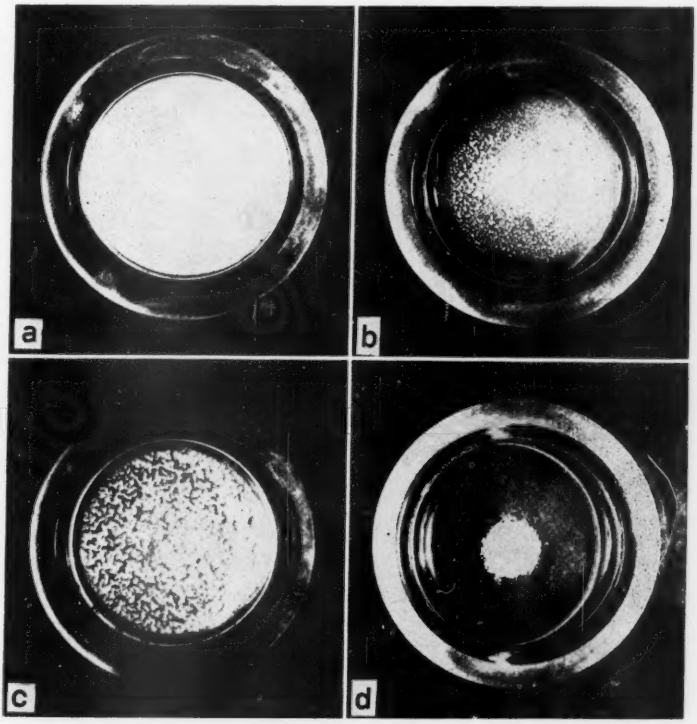

FIG. 1. Macroscopic appearance of agglutination reaction in the keyhole limpet, *Megathura crenulata*. Photographed in Syracuse dishes, × ½. *a*, Untreated sperm suspension (ca. 2 per cent.); *b*, *c*, and *d*, 15 seconds, 30 seconds and 10 minutes, respectively, after addition of solution of fertilizin.

clump of heads lined up like chromosomes on an equatorial plate and the tails united at their ends like spindle fibers. It certainly looks like a mitotic figure, but no matter how many times I show the figure, it never divides. I have discussed the question of head and tail agglutination in an

early publication and shall not take the time to go over the subject again here.

When a solution of antifertilizin, prepared from sperm or

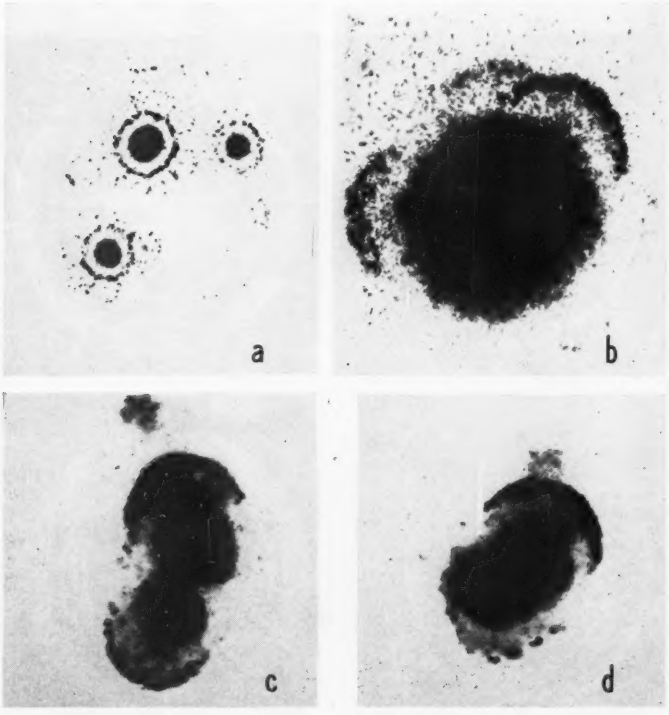

FIG. 2. Photomicrographs of agglutinated sperm of the keyhole limpet, *Megathura crenulata*. *a*, Three agglutinates formed in a moderately strong solution of fertilizin, showing spherical shell of sperm heads surrounding central mass of sperm; × 60. *b*, An agglutinate formed in a strong solution of fertilizin, showing incomplete shells of sperm heads attached to main mass by the ends of the tails; × 200. *c* and *d*, Fusion of two agglutinates; *d* was photographed 15 seconds after *c*; × 100.

from the interior of the eggs, is added to a suspension of intact eggs, then the eggs are agglutinated. Fig. 4 shows the microscopic appearance of the reaction when antifertilizin is added to a suspension of eggs of the sea urchin.

Fig. 5 shows some microscopic details of the action of antifertilizin on eggs of the sea urchin. Very soon after the

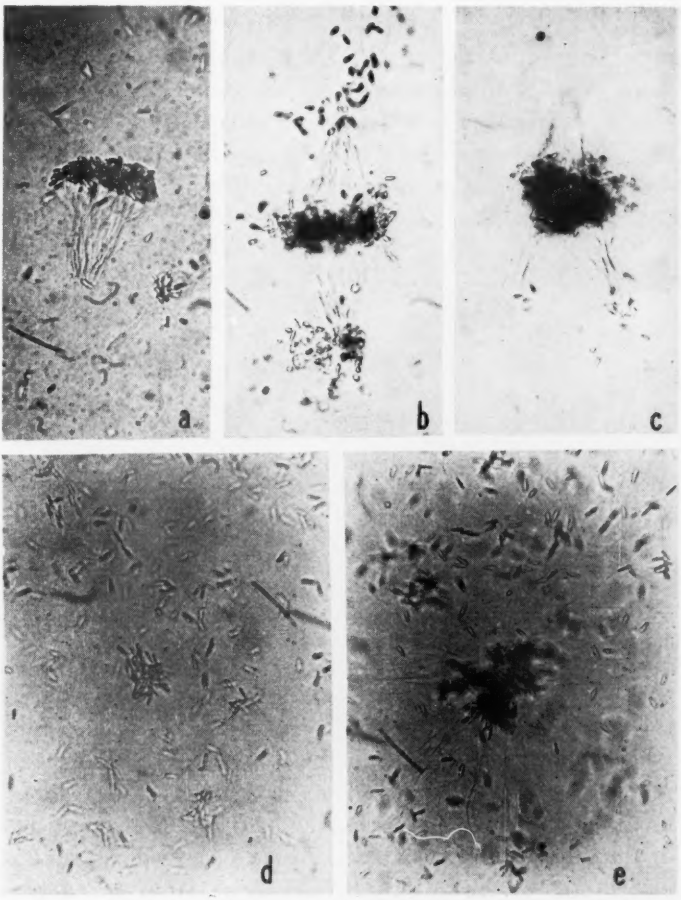

FIG. 3. Photomicrographs of sperm of *Megathura crenulata*, × 300. *a*, *b*, and *c*, Show union by heads and by end pieces of tails in strong, moderate and weak fertilizin solutions, respectively. *d* and *e*, Show head aggregates originally present in sperm suspension.

addition of a strong solution of antifertilizin a precipitation membrane is formed on the surface of the gelatinous coat

of the egg. The membrane thickens and contracts. In a strong antifertilizin solution it finally contracts to the surface of the egg and is then practically indistinguishable from the egg surface (Fig. 5*d*). This will take place in as short time as a minute in highly concentrated solutions. Some workers abroad interpreted the disappearance of the gelatinous coat as due to the action of a dissolving agent in the sperm extract. However, it is easy to demonstrate, by shaking off the gelatinous coat and adding the sperm extract

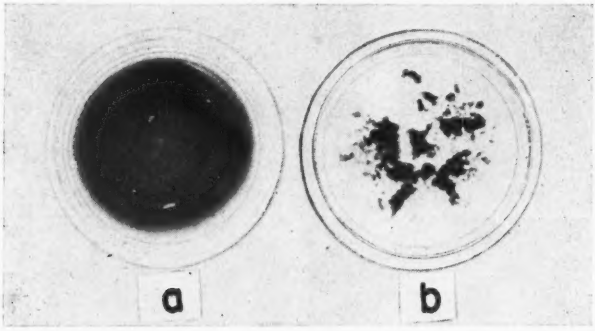

FIG. 4. Eggs of the sea urchin, *Strongylocentrotus purpuratus;* photographed in Syracuse dishes, × 7/10. *a,* Untreated egg-suspension. *b,* 15 minutes after addition of a solution of antifertilizin.

to the isolated coat, that it doesn't disappear. It persists in the form of the precipitation membrane. Figs. 5*e* and *f* are two photographs, in the time interval of 19 minutes showing the precipitation membrane shrinking to the surface of an egg and, in an adjacent isolated jelly hull, the persistence of the precipitation membrane. Figs. 5*g* to *j* illustrate the effect of antifertilizin on fertilized eggs. Here, too, a precipitation membrane forms, and it is interesting to note that it resembles the fertilization membrane that is present below it.

Fig. 6 illustrates the action of the lytic agent obtained from the sperm of mollusks; in this case from the keyhole

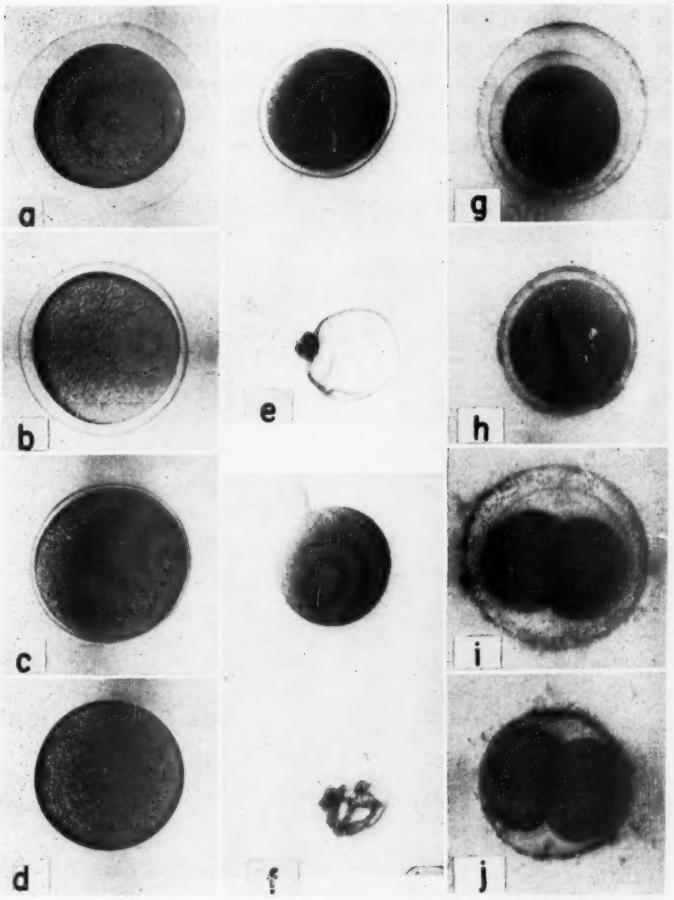

FIG. 5. Eggs of the sea urchin, *Lytechinus pictus*. *a*, *b*, *c*, and *d*, Successive pictures of the same egg at about ½ minute intervals after addition of a solution of antifertilizin, showing formation of the precipitation membrane and its contraction to the surface of the egg; × 400. *e* and *f*, Successive pictures of the same egg and an adjacent isolated jelly-hull at 1 minute and 20 minutes respectively after addition of antifertilizin, showing the persistence of the material of the isolated jelly-hull at the time when the precipitation membrane has contracted to the egg's surface and has become indistinguishable from it; × 350 *g* and *h*, Successive pictures of the same fertilized egg at 1 minute and 3 minutes after addition of antifertilizin. *i* and *j*, Successive pictures of a fertilized egg in the two-cell stage at 1 minute and 4 minutes after addition of antifertilizin; × 350.

limpet. The unfertilized egg of the keyhole limpet normally has a tough membrane on the surface. When the eggs are shed into the sea water the membrane separates somewhat from the surface of the egg proper. Upon the addition of the lysin-containing solution of sperm extract of

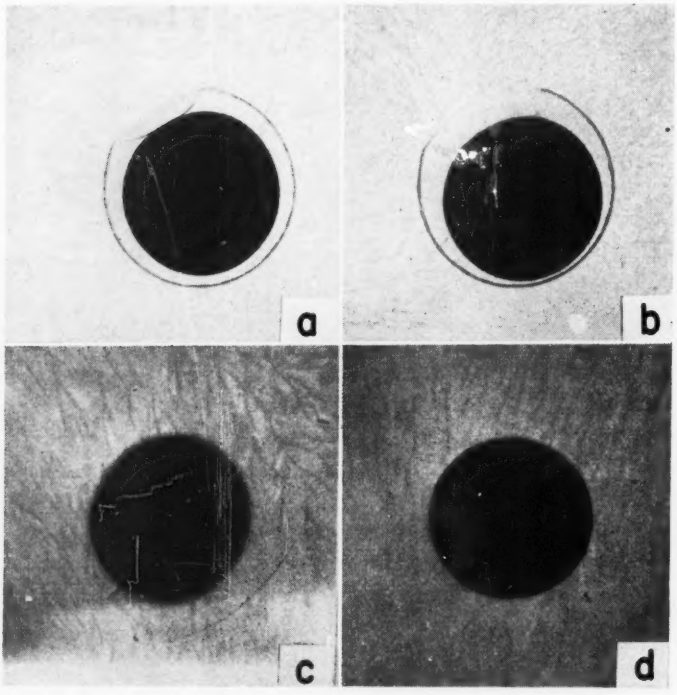

FIG. 6. Photomicrograph of an egg of *Megathura crenulata* at: *a*, 1 minute; *b*, 1¾ minutes; *c*, 2½ minutes; *d*, 3¼ minutes after addition of a sperm extract containing the egg-membrane lysin. × 200.

the keyhole limpet this membrane swells and thins out and finally disappears. This can take place in as short a time as forty-five seconds in a strong solution of lytic agent. Incidentally, the membrane is a rather tough affair and rather resistant to ordinary chemical treatment. Mr.

Krauss, who has been working with it recently in our laboratory, informs me that the eggs can be left in eight normal hydrochloric acid overnight with no noticeable effect on the membrane.

Specificity

One of the problems of fertilization in which we are interested is the species- and tissue-specificity of the process. In this connection we have made extensive tests of the cross-reactivity of fertilizin and antifertilizin of various species of sea urchins and starfish and have compared the degree of cross-reactivity with the degree of cross-fertilizability of the same species of animals.

First I should remark that we have some earlier evidence concerning the role of these substances in fertilization. For example, there are experiments in which the gelatinous coat of the egg is removed and experiments in which we deprive the sperm partly of the antifertilizin or treat them with antibodies against antifertilizin. These experiments show that fertilizin serves as an aid to fertilization, but only when it is present on the surface of the egg, as the gelatinous coat. If a concentrated solution of fertilizin is added to a suspension of eggs and fertilization is attempted, or if sperm are treated with fertilizin and used for insemination, fertilization is inhibited and may even be completely blocked. Evidently when the interaction of the sperm and fertilizin takes place away from the egg and is completed before the sperm reaches the surface of the egg, the sperm are then unable to combine with the egg. This is not due to the sperm being tied up in the agglutinates, because agglutination is spontaneously reversible in the sea urchin. After a period of agglutination, the length of which is dependent upon the concentration of fertilizin, the sperm separate, appear quite normal and swim actively about. Nevertheless, they are incapable of fertilization.

While eggs that have been deprived of their gelatinous coat show, in general, a reduced fertilizability, they are still fertilizable. This does not, however, mean that fertilizin,

as the gelatinous coat, serves only as an aid to fertilization and is not an essential link in the process. To decide whether or not it is essential for the process it would be important to remove the fertilizin completely. However, this does not appear to be readily feasible since, at the surface of the egg, where the gelatinous coat joins the egg, there is evidently a layer of fertilizin that is not removed by the treatments used to remove the gelatinous coat and which apparently can not be removed without disruption of the egg.

Similarly, the experiments on partially depriving sperm of antifertilizin show that their fertilizing capacity is impaired without corresponding impairment of their activity or their respiratory rate. Specific antibodies against antifertilizin can also impair the fertilizing power of the sperm without visible effect on the sperm.

In comparing the degree of cross-reactivity of these substances with the degree of cross-fertilization, there are a great many ways one may proceed, and I can, in a limited time, simply indicate one type of comparison and a sample of the data we have obtained. In the first place all the species of echinoids that we have tested here cross-fertilize. One can specify a degree of cross-fertilizability since the percentage of cross-fertilization that is obtained depends upon the amount of sperm employed for insemination. Of course, the percentage of fertilization obtained within the species, which we may call iso-fertilization, also depends upon the amount of sperm employed. This is apart from the fact that only one sperm enters the egg. One sperm is, of course, sufficient to fertilize an egg but because of several known, as well as unknown, factors, many more than one spermatozoon per egg must be present in the inseminates. As the amount of sperm employed in iso-insemination is increased the percentage of fertilization increases from zero to 100. In cross-fertilization the same thing occurs, except that it will require much higher concentrations of sperm and, in some cases, we may not be able to get the sperm concentrated enough to fertilize all the eggs. The degree of

cross-fertilizability of a particular pair of species can be expressed in terms of the amount of sperm required to give a certain percentage of fertilization below 100 per cent., say, five per cent., under standard conditions. Due to variations in different lots of animals, changes in activity of the sperm with time and similar factors it is advisable that as many different tests as possible be performed at the same time, or, at least, that there be a standard of comparison, for example, the iso-inseminations, in each set of tests. The degree of fertilizability of a particular combination can, then, be expressed by the figure for the number of times that a unit volume of standard sperm suspension is diluted in order to give the end point percentage of fertilization.

In Table 1 the average values for some of the data of this sort, accumulated for four species of echinoids, is presented

TABLE 1

(Comparison of Cross-Fertilization with Cross-Agglutination Among Echinoids) (The Upper Figures of Each Pair of Rows Represent the Number of Times the Sperm Suspension Is Diluted in Giving the End Point Value (2 per cent.) of Fertilization Under Certain Standard Conditions. The Lower Figures Are the Agglutination Titers in Terms of the Highest Dilution of Fertilizin Solution that Gives Visible Agglutination)

Eggs or Fertilizin of	Spermatozoa of			
	S. purp.	S. fran.	L. pictus	D. excent.
Strongylocentrotus purpuratus	3000	3	1	5
	512	8	64	4
Strongylocentrotus franciscanus	1	600	1	2½
	0	512	4	0
Lytechinus pictus	2	4	850	2½
	64	32	64	8
Dendraster excentricus	1½	40	2	4400
	4	2	1	128

as the upper figures in each pair of rows. The lower figures in each pair of rows give averages of the titers for the agglutination of sperm by fertilizin among the same species. No matter what basis of comparison we have used for these figures, or other data of this sort, we can find no very close correspondence between the degree of cross-fertilizability and the degree of cross-agglutination. We can interpret this to mean that the specificity of the fertilizin-antifertilizin reaction does not, in itself, account for the specificity of fertilization. This is not surprising, since there are un-

doubtedly many other reactions involved in the process of fertilization. Other, less extensive, tests with the lytic agent obtained from sperm of various mollusks show a broad cross-reactivity for this material which, likewise, can not by itself account for the species-specificity of fertilization.

On the other hand, emphasis should be placed upon the fact that where cross-fertilization occurs there is generally cross-agglutination. In Table 1 the only exception to this are the cases of *S. franciscanus* eggs or fertilizin with *S. purpuratus* and *D. excentricus* sperm. But these are only apparent exceptions, because we have found, by absorption experiments, that *S. purpuratus* sperm and *D. excentricus* sperm combine with *S. franciscanus* fertilizin. On the other hand, tests with various species of asteroids and other more distantly related animals, not listed in the table, have given neither cross-fertilization, cross-agglutination or absorption of the fertilizin by the foreign sperm.

Concerning tissue-specificity it has been shown by Lillie, and confirmed by various workers including ourselves, that no tissues other than the eggs and sperm yield fertilizin and antifertilizin.

We may, then, conclude that both the tissue and species specificity of fertilization is based, at least partly, on the specificity of the fertilizin-antifertilizin reaction.

We have also prepared antisera in rabbits against the various substances, including fertilizin and antifertilizin, obtained from the eggs and sperm of several species of echinoderms, annelids and mollusks. While a considerable amount of data has been accumulated, much more work will be required before a proper analysis can be made. I shall indicate briefly some of the results that have been obtained. In the first place the results show that the rabbit does not distinguish very closely the substances of one species of echinoid from those of another species. For example, as shown in Table 2, there is considerable cross-reactivity between an antiserum against the sperm extract of one species and the sperm of other species. Upon absorption one can demonstrate the presence of specific antibodies for different

TABLE 2

AGGLUTINATION TITERS OF RABBIT ANTISERA *vs.* THE ANTIFERTILIZIN OF THE SEA URCHINS *Strongylocentrotus purpuratus* AND *Lytechinus pictus*, TESTED ON SPERM OF VARIOUS SPECIES OF ECHINODERMS

Sperm of:	Antiserum vs. antifertilizin of	
	S. purp.	L. pictus
Echinoids		
Strongylocentrotus purpuratus	512	256
Strongylocentrotus franciscanus	512	128
Lytechinus pictus	128	128
Lytechinus anamesus	128	128
Lovenia cordiformis	8	16
Dendraster excentricus	16	16
Asteroids		
Patiria miniata	0	0
Pisaster ochraceous	0	0
Astropecten armatus	0	0

species, and the absorption tests indicate that there are at least two kinds of antibodies produced in response to the injection of what may represent a single molecular species. In the experiments listed in Table 3, for example, we have injected the rabbits with an electrophoretically homogeneous solution of antifertilizin of *S. purpuratus* and then absorbed the antibodies with the foreign sperm. That still leaves antibodies in the serum that react with the original sperm. If the antiserum is absorbed with homologous eggs it still agglutinates homologous sperm, but the titer is reduced. If absorbed with blood, it is reduced likewise, but in both of the latter cases the foreign sperm is agglutinated with unchanged titer. If it is absorbed with a mixture of the homologous blood and foreign sperm, then the antibodies against the homologous sperm are completely removed. In other words, after injection of antifertilizin of

TABLE 3

AGGLUTINATION TITERS OF AN ANTISERUM *vs.* ANTIFERTILIZIN OF *Strongylocentrotus purpuratus* AFTER ABSORPTION WITH VARIOUS KINDS OF CELLS

Absorbed with:	Tested on sperm of:	
	S. purp.	L. pictus
Unabsorbed	512	128
L. pictus sperm	128	0
L. pictus eggs	512	128
L. pictus blood	512	128
S. purp. eggs	256	128
S. purp. blood	256	128
S. purp. blood + L. pictus sperm	0	0

one species, there are antibodies formed that react with sperm of various species and also antibodies formed that react with other tissues (eggs and blood cells) of the same species but not with the corresponding tissues, the eggs and blood, of the foreign species. It might appear, then, that the injection of a single kind of protein molecule can give rise to antibodies that react with the same kind of protein in other species and also to antibodies that react with different proteins of the homologous species. Results of this sort that have been reported in the immunological literature have, in general, been interpreted as due to the presence of other protein of the homologous species as contaminants in the preparation used as the immunizing antigen. Further work will be required to determine whether or not this is true in our experiments as well as in some of the recent experiments with "purified" serum proteins that have given somewhat similar results.

Location of Antifertilizin and of the Lysin in the Spermatozoon

Mention was made earlier of the fact that fertilizin is derived from the gelatinous coat of the egg. We have also attempted to determine from what region of the sperm the antifertilizin is derived. To investigate this we have made use of the high magnification that is provided by the electron microscope. If sea urchin sperm is treated in such a way as to extract some antifertilizin, for example at pH 3.5, the head of the sperm between the acrosome and midpiece becomes spherical and slightly swollen, as shown in Fig. 7*b*. More drastic extraction results in further swelling and diffuseness, as in Fig. 7*c*. The acrosome is still present but covered by the swollen part of the head in this picture. Upon still further extraction the acrosome, mid-piece and tail remain intact, but the intervening region of the head becomes quite swollen and diffuse, as shown in Fig. 7*d*. We conclude that the antifertilizin is located laterally around the head, in the region between the acrosome and mid-piece.

We have also attempted to determine what part of the

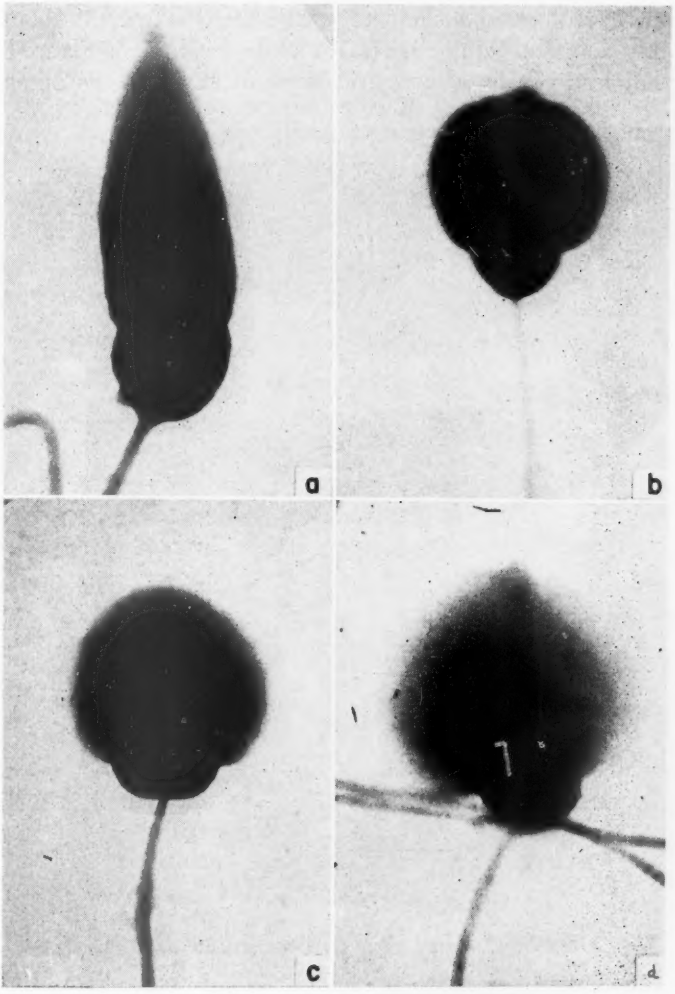

FIG. 7. Electron micrographs of sperm of the sea urchin, *Lytechinus pictus*, showing changes upon extraction of antifertilizin; × 30,000. *a*, Control, unextracted sperm. *b*, Extracted at pH 3.5. *c*, Extracted at pH 3.0. *d*, Extracted at pH 2.8.

sperm of the keyhole limpet contains the lytic agent. About this we are not quite as certain as the pictures of Figure 8 may lead you to believe. When we extract keyhole limpet

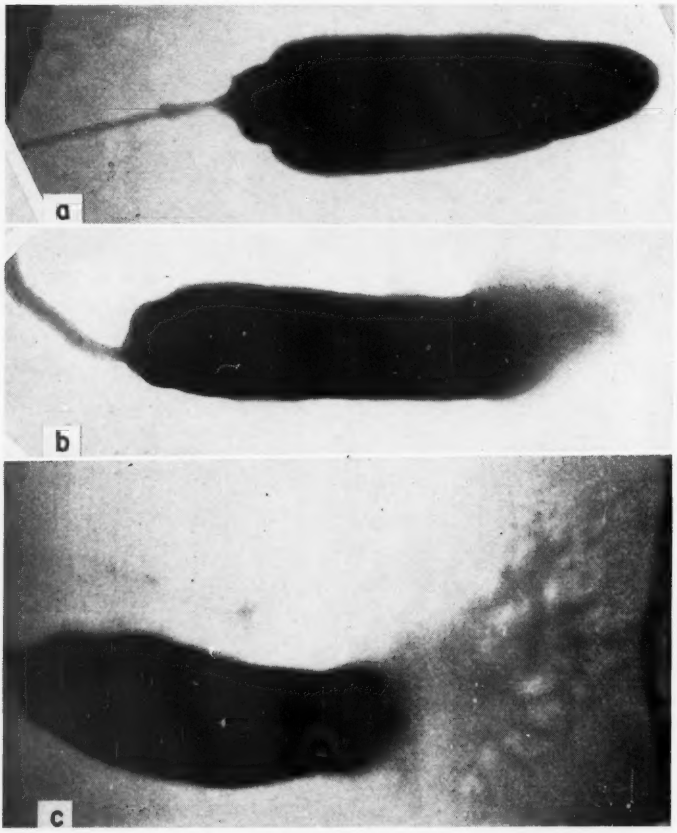

FIG. 8. Electron micrographs of sperm of the keyhole limpet, *Megathura crenulata*, showing breakdown of acrosome possibly correlated with liberation of egg-membrane lysin. *a*, Control sperm from a suspension in ordinary sea water. *b* and *c*, Sperm from a suspension in sea water at pH 9. × 30,000.

sperm in such a way as to obtain the lytic agent, we find the acrosome to be affected. Thus Figs. 8*b* and *c* show striking effects on the acrosome which may be broken down partially

or quite completely after an extraction with weak alkaline sea water that yields a solution of the lysin. The reason that I say we are not quite certain about this is that we find this sort of broken-down acrosome in untreated sperm too. While untreated sperm also yields some lysin in the supernatant, it is very difficult to make counts of the various types of sperm under the electron microscope in order to determine whether or not the number of disintegrated acrosomes might account for the amount of lysin obtained from untreated suspensions.

Chemical Properties

A fair amount of information has now been accumulated concerning the chemical nature of these various substances; especially the fertilizin and antifertilizin of sea urchins. Both of these can be prepared in electrophoretically homogeneous form. The antifertilizin is an acidic protein, isoelectric at about pH 3 and having a rather typical nitrogen content of about 16 per cent. Table 4 lists some electrophoretic mobility data obtained with the antifertilizins of Echinocardium by Runnström in Sweden and of Lytechinus

TABLE 4

Electrophoresis of Purified Solutions of Antifertilizin of *Echinocardium cordatum* (Runnström et al., 1942) and of *Lytechinus pictus* (Tyler, Unpub.)

pH	Buffer	Mobility in cm.²/sec./volt × 10^5	
		Echinocardium	Lytechinus
4.0	acetate	3.0	
4.5	acetate		4.2
4.6	acetate	3.7	
4.9	acetate		4.8
5.0	acetate	4.1	
6.0	phosphate		6.1
6.9	phosphate	6.4	
7.3	barbital		9.3

TABLE 5

Method of Preparation and Initial Purification of Fertilizin of the Sea Urchin *Strongylocentrotus purpuratus*

Extract 1 vol. of washed eggs with 40 vols. of pH 3.5 sea-water; agglutination titer = ca. 1000.
Absorb on sea-water salts precipitated by 4 ml N/1 NaOH per liter.
Dissolve in 3.3% NaCl and dialyze.
Precipitate with 1¼ vols. 95% Alc.
Dissolve in saline; reprecipitate with alcohol or ammonium sulphate.
Yield = ca. 250 mg./liter.

by ourselves. The two sets of values show reasonably good agreement.

Fertilizin is, in some respests, a more interesting substance, chemically. Table 5 gives a simplified method for the preparation of electrophoretically homogeneous fertilizin and Table 6 lists data for the electrophoretic mobility at

TABLE 6

ELECTROPHORESIS OF A PURIFIED SOLUTION OF FERTILIZIN OF THE SEA URCHIN *Strongylocentrotus purpuratus*

pH	Buffer	Mobility in cm.²/sec./volt × 10^5	
		Descending arm	Ascending arm
8.58	barbital	16.7	16.6
8.58	barbital	16.7	16.2
6.75	phosphate	....	18.8
6.60	phosphate	19.5	19.7
5.65	acetate	17.4	17.8
3.76	acetate	17.8	18.5
2.00	phosphate	.....	18.8

various pH's. As may be seen from the latter values the mobility changes very little as the pH is lowered from 8.6 down to 2.0. This means that fertilizin is a highly acidic substance. We now understand the basis of the highly acidic properties. Vasseur (1947), in Runnström's laboratory, has found about 25 per cent. of sulphate to be present, and we have confirmed this. Evidently we are dealing with a sulphuric ester, such as is found in chondroitin sulphate or mucoitin sulphate. We have also determined sedimentation constants of fertilizin preparations with a Pickel's analytical ultracentrifuge. It is relatively easy to obtain preparations, by a single careful extraction of the eggs, that are homogeneous in the ultracentrifuge. Fig. 9 shows the sedimentation boundary of one such preparation. This gives a sedimentation constant (S_{20}) of 6.3×10^{-13}, which, if the molecule were spherical, would correspond to a molecular weight of 82,000. After various precipitation procedures a large fraction of the material may become aggregated into larger particles which are poly-disperse in the ultracentrifuge.

Table 7 presents some of the analytical data obtained with purified fertilizin of *S. purpuratus*. In the first place it should be noted that fertilizin gives both protein and polysaccharide reactions; so it belongs to the group of sub-

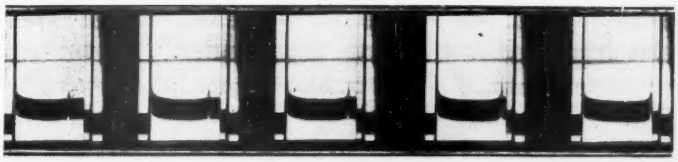

FIG. 9. Sedimentation diagram of a fertilizin preparation (pH 3.5 extraction and dialysis) of the sea urchin, *Strongylocentrotus purpuratus*, at 40,800 r.p.m and 6 cm. radius. The five photographs are taken at (reading left to right) 5, 21, 37, 53 and 69 minutes of centrifugation. The preparation shows a single boundary (peak) which sediments with an average s_{20} of 6.3×10^{-13} cm./sec./dyne/gm.

stances that are termed glycoproteins or mucopolysaccharides depending upon whether the protein or polysaccharide properties are considered to be strongest. It should, however, be emphasized that it does not appear to be possible to dissociate fertilizin into protein and polysaccharide fractions. We have made extensive attempts to do this and interpret the results to mean that protein and polysaccharide constituents do not exist as such in the molecule but rather that the various sugars and amino-acids are interlinked. As the figures of Table 7 show, values of about 25 and 20 per cent. have been obtained for reducing sugar and amino-acid respectively. These are minimum values, since a large amount of humin-residue is formed upon the acid hydrolysis of the fertilizin and an unknown amount of reducing sugar and amino-acid (tryptophane and perhaps tyrosine) is tied up in this residue. One of the sugars, that has been identified in the form of the osazone, is galactose.

TABLE 7

CHEMICAL ANALYSIS OF PURIFIED PREPARATIONS OF FERTILIZIN OF THE SEA URCHIN *Strongylocentrotus purpuratus*

	Per cent.
Nitrogen	5.6-5.8
Carbon	33.3
Hydrogen	5.5
Sulphate	23
Phosphate	0.06
Reducing sugar	$>$ 25
Amino-acids	$>$ 20
Glucosamine (?)	1.6
Galactose	pos.
Glucuronic acid	neg.
Pentoses	neg.
Mol. Wt. (as sphere)	82,000

Little, if any, real hexosamine is present, and tests for glucuronic acid and pentoses are negative. Upon paper chromatography of hydrolyzed fertilizin we find seven amino-acid spots. Table 8 lists the choices of particular amino-acids for each of these spots on the basis of their R_F values.

TABLE 8

CHROMATOGRAPHY OF A HYDROLYZED PREPARATION OF PURIFIED FERTILIZIN OF THE SEA URCHIN *Strongylocentrotus purpuratus* BY THE FILTER-PAPER METHOD OF CONSDEN, GORDON AND MARTIN (1944)

Spot No.	R_F values		Possible amino acid	R_F values	
	phenol	collidine		phenol	collidine
1	0.34	0	aspartic	0.30	0
2	0.43	0	glutamic	0.43	0
			serine	0.43	0
			α-amino-adipic	0.44	0
3	0.54	0	glycine	0.50	0
			threonine	0.57	0.03
			cystine	0.62	0
4	0.71	0	lysine	0.71	0
			alanine	0.70	0 01
5	0.82	0	histidine	0.77	0
			norleucine	0.84	0
			leucine	0.87	0
			arginine	0.87	0
6	0.85	0.09	tryptophane	0.80	0.12
			methionine	0.84	0.12
			phenylalanine	0.86	0.12
7	0.90	0.19	isoleucine	0.87	0.12

Sea urchin fertilizin belongs, then, to that group of sugar and amino-acid containing compounds that include such interesting substances as the human blood group substances. We have made some preliminary investigation of the composition of the fertilizins of different species of sea urchins. The present data show no qualitative difference between the two species *Strongylocentrotus purpuratus* and *Lytechinus anamesus*. It is known that antibodies with diverse reactivity may have the same chemical composition, the differences in specific reactivity being attributed to differences in shape of the molecule. The present data are too inadequate to indicate whether or not this may be true, too, of the fertilizins.

LITERATURE CITED

Consden, R., Gordon, A. H., and Martin. A. J. P.
1944. *Biochem. Jour.*, 38: 224–232.

Frank, J. A.
1939. *Biol. Bull.*, 76: 190–216.

Lillie, F. R.
1913. *Jour. Exp. Zool.*, 14: 515–574.
Runnström, J., Tiselius, A., and Vasseur, E.
1942. *Arkiv. Kemi, Mineral., Geol.*, 15A: No. 16, 1–8.
Tyler, A.
1939. *Proc. Nat. Acad. Sci.*, 25: 317–323.
1940. *Proc. Nat. Acad. Sci.*, 26: 249–256.
1947. *Growth*, 10 (Suppl): 7–19.
1948. *Physiol. Rev.*, 28: 180–219.
Vasseur, E.
1947. *Arkiv. Kemi, Mineral., Geol.*, 25B: No. 6, 1–2.

Discussion

Chairman Engle: Thank you, Dr. Tyler. You have extraordinarily important work before you. We will give the chemists a chance to discuss this.

Mr. MacGinitie: Was there any indication of recovery of the sperm after they had been, not inactivated, but put out of commission by fertilizin?

Dr. Tyler: No. In the sea urchin the agglutination reaction is a spontaneously reversible affair, such as it is, for example, with red blood cells agglutinated by influenza virus. When the agglutinates break up the sperm are perfectly normal in appearance and quite active. In fact, they are somewhat more active than previously, since there is an activating agent present in the egg water about which we don't know very much as yet. Nevertheless, they are incapable of fertilizing the eggs, and don't recover this ability on aging.

Mr. MacGinitie: What made me ask was because of the experiment done on mixing sperm with the eggs in the Urechis segmental organ or storage organ. There is no fertilization within the organ, but after removal of the mixture to sea water the sperm begin to recover and will fertilize eggs in time.

Dr. Tyler: I think that is a very interesting and important finding in regard to fertilization. There has been a good deal of earlier work, as you know, on the so-called blood-inhibition of fertilization in sea urchins. Dr. Pequc-

gnat recently went over that work again. Perhaps he would like to say a few words about it.

DR. PEQUEGNAT: At present it is possible to say a substance obtained from certain blood cells of the perivisceral fluid of several echinoderms (especially echinoids) is capable of blocking fertilization. As yet one can not say with certainty that the substance operates against the egg, but there is more evidence in favor of this opinion than for the opinion that it is the sperms which are inactivated. Assuming that the substance does act principally upon the egg, it is of considerable interest that the blocking effect is reversible. Thus, eggs previously exposed to the inhibitor and thereby rendered unfertilizable could be rendered fertilizable by simply washing them in several changes of sea water. At present I am attempting to determine the mechanism responsible for this blocking of the egg.

DR. TYLER: As I recall, you obtained it also from the tube feet of the animal. Is that correct?

DR. PEQUEGNAT: Yes. I found a very rich source of inhibitor to be certain yellowish bodies (presumably one type of amoebocyte) in the hypodermis of the oral tube feet. Although the generally held opinion regards these cells as being laden with waste products (at least one of which is very likely the inhibitor itself), one can not escape the conviction that such is not the case. For it seems very unlikely that a large concentration of waste products derived from another point in the body would be stored in the most active organs of the animal.

MR. MACGINITIE: The experiment I was referring to was the one in which the sperm was placed in the storage chamber and left there for a period of twenty-four hours. Then after they were taken out they appeared to be just as active as sperm could be but would slide all over the surface of an egg without attaching. After a period of an hour some would begin to attach, and from then on fertilization could take place.

DR. TYLER: The reason that I brought up blood inhibi-

tion in this connection is that it seems quite likely that the fluid within the storage organs is very much like the plasma, since these organs are in direct communication with the hemocoel. As Dr. Pequegnat has shown, the so-called blood inhibition is reversible upon washing the gametes in sea water. So it is conceivable that, in your Urechis experiments, the dilution occasioned by transfer of the mixture of eggs and sperm to sea water permits the inhibiting agent, which may be loosely absorbed on the surface of the gametes, to diffuse slowly away and thus become ineffective.

MR. MACGINITIE: you don't feel that it was the fertilizin or the contact with the egg, or that the activity of fertilizin had anything to do with it?

DR. TYLER: Of course I would like to interpret everything on the basis of fertilizin, but that temptation is just too great. I will have to resist it in cases like this until there is definite evidence that fertilizin is directly involved.

DR. HAAS: Hyaluronidase in snake venoms or in bacterial extracts is accompanied by other enzymes which we have designated as proinvasins. Are there any indications to suggest that the lytic substance in sperm might be associated with other proinvasin-like enzymes?

DR. TYLER: I can say it could be. The lytic agent has not, as yet, been really purified. So it is possible that two or more substances may be involved. One experiment which relates to the question consists in adding heat-inactivated sperm extract to fresh sperm extract. This does not result in increased activity. But this does not necessarily mean that only one substance is involved, since the other substances might also be heat-labile or the heat-labile component might be normally present in excess.

DR. HAAS: Would it be possible, for example, to add small amounts of snake venom to fertilizin and to obtain thereby an increased activity of the lytic substance?

DR. TYLER: I don't know whether we have any data that would properly answer your question. Mr. Krauss, do you know?

DR. MAX KRAUSS: In the work that I have been doing here with the egg membrane lysin in the keyhole limpet it is my impression, at this time at least, that there is no other substance which acts with it in the manner which you suggest.

DR. LATIES: How pertinent is the supposition that the areas found disrupted on the sperm as a result of extraction are necessarily the sources of the substance you are seeking?

DR. TYLER: I presume that you are asking whether or not I have any rational basis for the conclusion other than my inference from the pictures. First, I think it is rational to assume that the antifertilizin is somewhere on the surface of the sperm by virtue of the fact you get agglutination by adding fertilizin. There have been claims of action at a distance in antigen-antibody reactions, such as in the work of Rothen, but these have not been substantiated and have been effectively contradicted by the recent experiments of Singer in the Chemistry Division here. Since we can safely assume that antifertilizin is on the surface we can interpret the figures as meaning that dissolving it off weakens the surface in such a way that that part of the head can swell. It is possible that other material might leak out of the inside of the head but, at least in our preparations, we don't find any indication of a second component.

DR. CHAMBERS: In regard to the role of fertilizin in fertilization, I am interested in your interpretation of this experiment. Arbacia eggs are placed in a non-electrolyte solution and then they are tested for nakedness of the protoplasmic surface by the oil-drop method. We found that the surface of the protoplasm met all the criteria of an entirely naked surface. When these eggs were placed in sea water containing sperm, they apparently went ahead and developed normally, having fertilized normally, of course with the absence of fertilization membrane. I am wondering what your interpretation would be.

DR. TYLER: We, too, had found, as I indicated earlier, that sea urchin eggs can be fertilized after removal of the

gelatinous coat. We have evidence to show that there is a layer of fertilizin left on the surface itself. I think Dr. Moore, this afternoon, will tell us some more about membrane inhibition.

CHAIRMAN ENGLE: If there are no more comments, we will continue. We shall proceed with further studies. Dr. Berg, of the University of California at Berkeley, will speak on "Some Effects of Sperm Extracts in the Mussel Mytilus."

(*Conference continued in next issue*)